

Nisin affects the growth of *Listeria monocytogenes* on ready-to-eat turkey ham stored at four degrees Celsius for sixty-three days

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The ability of nisin to inhibit the growth of *Listeria monocytogenes* on ready-to-eat turkey ham was evaluated. The results indicated that nisin had no inhibitory effect on *L. monocytogenes* at 4°C for up to 10 days. At 10°C, nisin inhibited the growth of *L. monocytogenes* for up to 20 days. At 20°C, nisin inhibited the growth of *L. monocytogenes* for up to 30 days. At 30°C, nisin inhibited the growth of *L. monocytogenes* for up to 40 days. At 40°C, nisin inhibited the growth of *L. monocytogenes* for up to 50 days. At 50°C, nisin inhibited the growth of *L. monocytogenes* for up to 60 days. These results indicate that nisin can be used to inhibit the growth of *L. monocytogenes* on ready-to-eat turkey ham for up to 60 days.

Keywords: *Listeria monocytogenes*; nisin; ready-to-eat turkey ham; storage

Listeria monocytogenes has been implicated in many foodborne outbreaks of disease in the United States and abroad (Bachman et al., 1994). This bacterium has been associated with a variety of food products, including deli meat, cheese, raw milk, and soft cheeses (Bachman et al., 1994). *Listeria monocytogenes* has also been implicated in foodborne outbreaks associated with processed meats, such as hot dogs, ham, and salami (Bachman et al., 1994).

Several factors have been implicated in the growth of *Listeria monocytogenes* on food products, including pH, water activity, temperature, and the presence of other microorganisms (Bachman et al., 1994).

Nisin is a peptide antibiotic produced by several species of lactic acid bacteria, including *Lactococcus*, *Lactobacillus*, and *Leuconostoc* (Klaenhammer, 1990).

Nisin has been shown to inhibit the growth of *Listeria monocytogenes* (Klaenhammer, 1990; Klaenhammer et al., 1990; Klaenhammer &

Wong, 1990). Nisin has also been shown to inhibit the growth of *Listeria monocytogenes* on various food products, including deli meat, cheese, and soft cheeses (Klaenhammer, 1990; Klaenhammer et al., 1990; Klaenhammer & Wong, 1990). Nisin has also been shown to inhibit the growth of *Listeria monocytogenes* on processed meats, such as hot dogs, ham, and salami (Klaenhammer, 1990; Klaenhammer et al., 1990; Klaenhammer & Wong, 1990).

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concentration of 10^4 cfu/g. Predetermined aliquots were inoculated, chopped, and placed in prelabeled vacuum bags (6.7 g and 0% RH; FoodSaver, T150 Corporation, Rye, NY). Six turkey samples containing either water or 10^4 cfu/g *L. monocytogenes* were prepared for each treatment. Each sample was cut into 10 pieces and randomly assigned to one of three vacuum bags. The bags were sealed and placed in a vacuum chamber at 100% RH and ambient temperature. The samples were held for 24 h and then placed in a walk-in chamber at 4°C for 14 d.

Data Analysis

A complete randomized block design was employed. A total of 240 samples were analyzed (i.e., duplicate samples, 6 treatments, 10 storage days, 2 trials). The general GLM program (PROC GLM) of SAS (Version 8.02, SAS Institute, Cary, NC) was employed to determine differences between treatments, storage days, and the interaction of treatment and storage day. Analysis of variance (ANOVA) was determined using SAS. Tukey's HSD test was used to determine which treatment(s) differed.

Table 2. pH measurements for ready-to-eat turkey ham treated with various concentrations of nisin, inoculated with *Listeria monocytogenes*, and stored at $4 \pm 1^\circ\text{C}$ for 63 d¹

Treatment	Days of storage									
	0	7	14	21	28	35	42	49	56	63
Negative	6.24 ^{a,x}	5.76 ^{a,y}	4.97 ^{a,z}	4.87 ^{a,z}	4.86 ^{a,z}	4.81 ^{a,z}	4.79 ^{a,z}	4.67 ^{a,z}	4.86 ^{b,z}	4.82 ^{b,z}
Positive	6.14 ^{a,x}	5.07 ^{a,y}	4.85 ^{a,y}	4.79 ^{a,y}	4.78 ^{a,y}	4.82 ^{a,y}	4.77 ^{a,y}	4.94 ^{a,y}	4.74 ^{b,y}	4.77 ^{b,y}
0.2% nisin	6.21 ^{a,x}	5.67 ^{a,xy}	5.46 ^{a,xy}	5.29 ^{a,xy}	5.19 ^{a,xy}	5.00 ^{a,y}	5.02 ^{a,y}	5.13 ^{a,xy}	5.01 ^{b,y}	4.86 ^{ab,y}
0.3% nisin	6.22 ^{a,x}	6.10 ^{a,x}	6.11 ^{a,x}	5.72 ^{a,x}	5.43 ^{a,x}	5.41 ^{a,x}	5.40 ^{a,x}	5.29 ^{a,x}	5.10 ^{b,x}	5.24 ^{ab,x}
0.4% nisin	6.26 ^{a,x}	5.85 ^{a,xy}	5.56 ^{a,xy}	5.41 ^{a,xy}	5.06 ^{a,y}	5.15 ^{a,y}	4.90 ^{a,y}	4.76 ^{a,y}	4.87 ^{b,y}	5.12 ^{ab,y}
0.5% nisin	6.21 ^{a,x}	6.14 ^{a,xy}	6.14 ^{a,xy}	6.08 ^{a,xy}	5.89 ^{a,xyz}	5.58 ^{a,yz}	5.79 ^{a,xyz}	5.67 ^{a,z}	5.80 ^{a,xyz}	5.83 ^{a,xyz}

^{a,b}Means within a column lacking a common superscript differ ($P < 0.05$).^{x,z}Means within a row lacking a common superscript differ ($P < 0.05$).¹Each mean value represents 8 individual measurements.

decrease per 10⁶ U/Morla et al., 2001) treated with nisin when compared to the positive control suggested that a common antimicrobial effect was being exerted by the nisin treatments.

es Analysis

genes counts remained at 4.14 to 4.16 log cfu/g through 63 d of storage for the positive control. This confirmed that the desired *L. monocytogenes* concentration of 4 log cfu/g was achieved, all nisin treatments reduced *L. monocytogenes* counts to the positive control on d 0, 7, and 14. When nisin was isolated on the negative control, it resulted in 4 log reductions in *L. monocytogenes* counts when compared to the positive control and 4 log reductions in treatment \times day interactions. This was primarily due to the *L. monocytogenes* counts on d 0, 7, and 14. Except for d 0, no significant reductions occurred for d 0 through 63 d of storage. The extended lag phase

Lactic Acid Bacteria Analysis

Lactic acid bacteria populations increased as storage time increased for all treatments (Table 4). On d 0 and 49, lactic acid bacteria counts were significantly lower ($P < 0.05$) for all nisin treatments when compared with the positive control. Except for d 7, 14, and 21, hams treated with 0.5% nisin had lower ($P < 0.05$) lactic acid bacteria counts when compared with the positive and negative controls. Lactic acid bacteria counts were also lower ($P < 0.05$) for hams treated with 0.3% nisin ($P < 0.05$) when compared with positive and negative controls on d 56. This observation revealed the antimicrobial properties of nisin against lactic acid bacteria. It was discussed earlier that the pH values increased as the concentration of nisin increased to 0.5%. The data suggested that the shelf life of vacuum-packaged cured RTE poultry products may be extended with the use of 0.5% nisin by suppression of the growth of lactic acid bacteria.

In conclusion, this study revealed that nisin can be successfully incorporated into RTE turkey ham to control *L. monocytogenes* and lactic acid bacteria. The ant-

L. monocytogenes Analysis

Listeria monocytogenes counts increased from 4.97 log cfu/g through 63 d of storage for the positive control, which controlled the *L. monocytogenes* inoculum concentration achieved (Table 3). In general, nisin treatments resulted in reduced ($P < 0.05$) *L. monocytogenes* counts when compared with the positive control. On d 0, all nisin treatments demonstrated reductions ($P < 0.05$) in *L. monocytogenes* counts compared with the positive control. The reductions were also observed on d 7 for 0.4% nisin, d 14 for 0.5% nisin, and d 21 for the 0.2% nisin treatment compared with the positive control. The reductions in *L. monocytogenes* were observed 21 d after samples treated with 0.5% nisin. The interaction ($P < 0.05$) was attributed to the increase ($P < 0.05$) in *L. monocytogenes* counts from d 42 through 63 for the 0.2% nisin treatment, the 0.2% nisin treatment on d 42 through 63, and the significant increase in *L. monocytogenes* counts for hams treated with nisin through 63 d. The 0.5% nisin treatment demonstrated an ex-

log cfu/g) for ready-to-eat turkey ham treated with various concentrations of nisin, inoculated with *L. monocytogenes*, and stored at $4 \pm 1^\circ\text{C}$ for 63 d¹

Treatment	Days of storage							
	0	7	14	21	28	35	42	49
Negative	0.00 ^{b,y}	0.00 ^{b,y}	0.00 ^{b,y}	0.00 ^{b,y}	0.00 ^{a,y}	0.00 ^{b,y}	0.00 ^{c,y}	0.00 ^{c,y}
0.2% nisin	4.97 ^{a,y}	4.92 ^{a,y}	4.95 ^{a,y}	4.50 ^{a,y}	4.23 ^{a,y}	4.32 ^{a,y}	4.18 ^{a,y}	4.14 ^{a,y}
0.3% nisin	0.97 ^{b,z}	2.12 ^{b,yz}	3.22 ^{b,yz}	2.45 ^{b,yz}	2.94 ^{b,yz}	3.35 ^{a,yz}	3.57 ^{a,y}	2.97 ^{b,y}
0.4% nisin	0.60 ^{b,y}	1.59 ^{b,y}	1.96 ^{a,y}	2.16 ^{a,y}	2.95 ^{a,y}	2.62 ^{a,y}	2.57 ^{a,y}	1.83 ^{cd,y}
0.5% nisin	0.60 ^{b,y}	0.85 ^{b,y}	2.29 ^{a,y}	1.35 ^{a,y}	2.50 ^{a,y}	2.96 ^{a,y}	3.06 ^{a,y}	2.09 ^{a,y}
Positive	0.42 ^{b,y}	0.89 ^{b,y}	0.17 ^{b,y}	1.66 ^{a,y}	1.90 ^{a,y}	1.95 ^{a,y}	1.67 ^{a,y}	1.01 ^{d,y}

within a column lacking a common superscript differ ($P < 0.05$).

within a row lacking a common superscript differ ($P < 0.05$).

Each mean value represents 8 individual measurements.

Table 3. *Listeria monocytogenes* counts (log cfu/g) for ready-to-eat turkey ham treated with various concentrations of nisin, inoculated with *L. monocytogenes*, and stored at $4 \pm 1^\circ\text{C}$ for 63 d¹

Treatment	Days of storage							
	0	7	14	21	28	35	42	49
Negative	0.00 ^{b,y}	0.00 ^{b,y}	0.00 ^{b,y}	0.00 ^{b,y}	0.00 ^{a,y}	0.00 ^{b,y}	0.00 ^{c,y}	0.00 ^{c,y}
0.2% nisin	4.31 ^{a,y}	3.52 ^{a,y}	3.10 ^{a,y}	2.82 ^{ab,y}	1.51 ^{bc,y}			
0.3% nisin	3.52 ^{a,y}	3.10 ^{a,y}	2.99 ^{ab,y}	2.82 ^{ab,y}				
0.4% nisin	3.10 ^{a,y}	2.82 ^{ab,y}	3.02 ^{ab,y}					
0.5% nisin	2.82 ^{ab,y}	1.89 ^{ab,y}	1.51 ^{bc,y}					

^{a,b}Means

^{x,y}Means

¹Each me

Table 4. Lactic acid bacteria counts (log cfu/g) for ready-to-eat turkey ham treated with various concentrations of nisin immediately with *Listeria monocytogenes*, and stored at 4 ± 1°C for 63 d

Treatment	Days of storage									
	0	7	14	21	28	35	42	49	56	63
Negative	3.25 ^{a,b}	4.74 ^{a,b}	5.66 ^{a,b}	6.86 ^{a,b}	7.10 ^{a,b}	6.84 ^{a,b}	6.66 ^{a,b}	6.71 ^{a,b}	6.62 ^{a,b}	6.66 ^{a,b}
Positive	4.27 ^{a,b}	5.49 ^{a,b}	5.90 ^{a,b}	6.75 ^{a,b}	6.61 ^{a,b}	6.74 ^{a,b}	6.54 ^{a,b}	6.47 ^{a,b}	6.53 ^{a,b}	6.71 ^{a,b}
0.2% nisin	1.55 ^{a,b}	3.60 ^{a,b}	4.18 ^{a,b}	4.65 ^{a,b}	4.43 ^{a,b}	5.28 ^{a,b}	5.43 ^{a,b}	5.57 ^{a,b}	5.65 ^{a,b}	5.12 ^{a,b}
0.3% nisin	1.99 ^{a,b}	2.44 ^{a,b}	2.79 ^{a,b}	3.46 ^{a,b}	3.94 ^{a,b}	4.95 ^{a,b}	4.96 ^{a,b}	4.31 ^{a,b}	4.52 ^{a,b}	4.94 ^{a,b}
0.4% nisin	1.28 ^{a,b}	2.73 ^{a,b}	3.43 ^{a,b}	3.86 ^{a,b}	4.80 ^{a,b}	5.44 ^{a,b}	5.18 ^{a,b}	5.66 ^{a,b}	5.29 ^{a,b}	5.11 ^{a,b}
0.5% nisin	1.32 ^{a,b}	1.92 ^{a,b}	1.59 ^{a,b}	3.07 ^{a,b}	3.13 ^{a,b}	4.20 ^{a,b}	4.19 ^{a,b}	4.23 ^{a,b}	3.48 ^{a,b}	3.00 ^{a,b}

^{a,b}Means within a column lacking a common superscript differ ($P < 0.05$).^cMeans within a row lacking a common superscript differ ($P < 0.05$).

Each mean value represents 8 individual measurements.

antimicrobial effectiveness of nisin increased as its concentration increased from 0.2 to 0.5% with the most effective antimicrobial level being 0.5%. The data revealed that 0.2, 0.3, and 0.4% nisin treatments resulted in lower ($P < 0.05$) *L. monocytogenes* when compared with the positive control initially (d 0) and for 1 wk (d 7 d), and on d 49. In comparison, the 0.5% nisin treatment resulted in lower ($P < 0.05$) *L. monocytogenes* counts initially (d 0) and for 2 wk (d 14 d), and on d 49 and 63. Although the counts on d 21, 28, 35, 42, and 56 were not significantly lower ($P > 0.05$) than the positive control, they were at least 2 log less than the positive control. The data for pH revealed that 0.5% nisin was effective in increasing pH of the ham and simultaneously decreasing lactic acid bacteria counts.

This study also revealed that the 0.5% nisin treatment exerted maximum antimicrobial effects on *L. monocytogenes* at 63 d of storage. This may be due to the fact that the

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